Stylet increment analysis in *Eledone cirrhosa* from the north-western Iberian population

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Summary
Specimens of *Eledone cirrhosa* (Lamarck, 1798) were obtained from trawling fishing undertaken from March 2009 to March 2010 in north-western Iberian coasts. Stylet Increment Analysis (SIA) was applied to 122 specimens ranging 67-950 g, representing the size classes detected by modal progression analysis performed to the catches collected throughout the year. Despite the significant correlation between size and age, modal progression analysis does not seem to provide an adequate vision of age-structure in this area. Assuming a daily deposition rate of growth increments in the stylet, the age of the specimens was estimated and compared with morphometric data of the animals and the stylets to determine the best morphometric predictor of age. Results showed differential longevity between sexes, with females reaching a life span of up to two years. Based on estimated age, hatching date was back-calculated, showing a spawning peak during winter. The recruitment of *E. cirrhosa* in Galicia waters would occur when animals reach 3-4 months old. Based on size at first maturity estimated for this species in this area and DML-Age relationships, females would achieve maturity at around one year old, while males would do it at approximately at 8-9 months old. The results obtained were discussed according to the scarce information available from the Iberian population.

Introduction
Age determination is necessary to understand and estimate various parameters that determine the biology of marine species, such as growth rate, population age structure, longevity, mortality, productivity or recruitment process. Recently, direct ageing methods based in reading growth increments deposited in hard structures have been developed to determinate age in octopods (Hernandez-Lopez et al. 2001; Perales-Raya et al. 2010; Canali et al. 2011). Stylet increment analysis (SIA) has been applied and daily growth increment deposition validated in *Octopus vulgaris* (Hermosilla et al. 2010), *O. pallidus* (Doubleday et al. 2006) and *Octopus maya* (Rodríguez-Domínguez et al., 2013), and have been proven to be a reliable method for determining age in octopods. The horned octopus *Eledone cirrhosa* (Lamarck, 1798) is a medium-size benthic octopod, widely distributed over the continental shelves of the north-eastern Atlantic and Mediterranean Sea. A 20 months lifecycle was postulated in Scottish waters (Boyle and Knobloch, 1982). However, this issue still remains unchecked mainly due to the lack of studies focusing ageing and population structure of the species. This species breeds throughout the year but typically shows a peak, which in the northwest of the Iberian Peninsula takes place between late spring and early summer (Regueira et al. 2014). Although commercial interest of E. *cirrhosa* increased during the last decade in north-western Iberian waters, the biology of this species remains poorly studied in this region. Our main objective is to estimate age and growth of this species using growth increment in stylets and obtain new insights on the annual cycle of *E. cirrhosa* in north-western Iberian Peninsula waters.

Materials and Methods
A total 2,220 individuals of *E. cirrhosa* were monthly collected in three Galician ports (north-western Iberian Peninsula) and later processed. Body weight (BW) and eviscerated body weight (EBW), and dorsal mantle length (DML) were annotated. Specimens were sexed according to an adaptation of Inejih (2000) maturity scale to assign four stages for males and five stages for females. Stylets of thirty specimens of each sex and fishing ground were monthly taken and stored in ethanol 70% until preparation. Based on previous studies on age validation performed in three octopus species (Doubleday et al. 2006; Hermosilla et al. 2010; Rodríguez-Domínguez et al. 2013) a daily deposition rate of growth increments in the stylet of *E. cirrhosa* was assumed. Thus, age of the specimens was estimated and hatching dates were back-calculated from capture date. Estimated age was then compared between sexes and fishing ground and relationship with specimens and stylet morphometry was checked.

Results and Discussion
The method for sample preparation described by Barrat & Allcock (2010) and applied herein provided durable samples and proved its suitability for ageing studies in *Eledone cirrhosa* (Figure 1). Our results show also that this

species hatch throughout the year in north-western Iberian waters, with a peak in January. According to the reproductive cycle of the species in this area, which showed a peak in June (Regueira et al., 2013), the elapsed time between maturity and maximum egg hatching would take about six months.

Embryonic development of *E. cirrhosa* at 16°C takes about 110 days (Mangold et al. 1971). Since sea bottom temperature in north-western Iberian continental ranges from 11°C to 14°C (Gago et al., 2011), an increase of time for embryonic maturation must be expected. Moreover, females may copulate several weeks before egg laying begins and could preserve the sperm for at least six weeks (Mangold et al. 1971). According to this and the results on estimated age, with a maximum of 517 days for females and 420 for males, the life span would be around two years, agreeing with the estimated for Scottish populations.

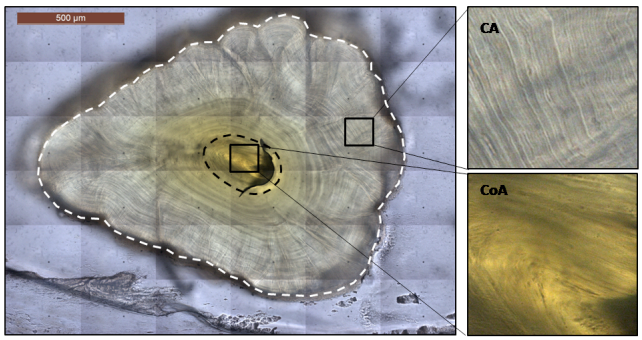

Figure 1. Composite image of an *Eledone cirrhosa* stylet preparation, showing the counted area (CA), in which growth rings are discernible, and the core area (CoA

Previous beak studies in *O. vulgaris* did not show a significant differential longevity between sexes (Hernandez-Lopez, 2001; Canali, 2011). Conversely, Leporati (2008) found significant differences in age between sexes by reading stylets in *O. pallidus*. This lack of uniformity in the published results could reveal inconsistencies in sampling methods, instead of a physiological fact in the species. Different catchability between sexes can occur, supposing females to perform parental care of the eggs, so they might not be captured during this period, accounting about four months, while males can be captured all throughout his life. On the other hand, *E. cirrhosa* females migrate over coastal areas to spawn (Regueira et al., 2014). During this reproductive migration, they could be more exposed to the fishery, while mature males would remain in deeper areas, outside the fishing grounds operated by commercial trawlers.

Based on the estimated ages, the recruitment to the fishery of *E. cirrhosa* in Galician waters would occur when animals reach 3-4 months old. This also agreed with fishery catch pattern of this species in north-western Galician waters, which capture peak of new recruits takes place around May (Regueira et al., 2014). Although a tendency is observed, the size class structure did not show consistency with the estimated hatching date of the specimens. Thus, hatching dates of individuals belonging to different size classes do overlap. Therefore, MPA did not to provide an adequate vision of age-structure in *E. cirrhosa*.

Based on size at first maturity estimated for this species in north-western Iberian coasts (Regueira et al., 2013) and DML-Age relationships in this paper, females would achieve maturity at around a year, while males would do at approximately 8 or 9 months. The results obtained in this study reinforces the importance of age validation to confirm our interpretations, not only because the deposition rate of growth increment could have a different periodicity, but also because the growth process is almost unknown during early life history stages and, as well as observed in statoliths the central area of the stylet may undergo compaction during early growth, which would make harder to estimate the number of rings in this area and could bias the results on estimated age (González et al., 2010). However, the concordance between the life cycle described for the species, the pattern of catches and the present interpretation of the population structure by age estimates, supports the validity of our conclusions.

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